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PHENOTYPIC SCREENING AND MOLECULAR CHARACTERIZATION OF CHICKPEA GENOTYPES AGAINST FOUR DIFFERENT RACES OF *FUSARIUM* WILT

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ABSTRACT

The present investigation was undertaken for phenotypic evaluation of six genotypes of chickpea *viz*. PG043, HC1, DCP92-3, GNG1581, PKG024 and PG071 for resistance to four Foc races (1, 2, 4 and 6) of chickpea wilt pathogen and their molecular characterization. Eight markers *viz*. four microsatellite based markers (TA110, TA59, TA96 and TA27) linked to resistance allele, 2 RAPD markers: UBC-170 and $A_{07}C_{417,}$ 1 SCAR marker: $CS_{27}A_{700}$ and 1 ISSR marker: UBC-825 linked to susceptibility, that have been already validated in diverse genetic backgrounds were taken for molecular screening. Amplified allele size for resistance and susceptibility obtained by each primer-genotype combination, were recorded. Genotypes PG043, GNG1581 and PG071 were found to be resistant for three races 1, 2 and 4, while PKG024 was found to be susceptible after phenotypic screening and molecular characterization with all the linked markers. The use of the sorted wilt resistant genotypes can make available ample prospect in MAS breeding of chickpea.

KEYWORDS: Artificial Screening, Chickpea, Fusarium wilt, Molecular Markers, Resistance

INTRODUCTION

Chickpea (*Cicer arietinum*, 2n=2x=16) is an edible self fertilizing annual diploid grain legume of the family Fabaceae, sub family Faboideae. It is the only cultivated species of the genus Cicer, that evolved from its wild progenitor *Cicer reticulatum* by selection. *Fusarium* wilt caused by the deuteromycetes fungal pathogen *Fusarium oxysporum* f. sp. *ciceris* is one of the major constraints in realization of the full yield potential (4 t/ha) of chickpea as pathogen can destroy the crop completely or cause significant annual yield losses. This disease causes huge yield losses (10 to 90%) annually (Singh and Reddy, 1991). Eight races of *F. oxysporum* f. sp. *ciceris* (Foc) have now been identified: 0, 1A, 1B/C, 2, 3, 4, 5 and 6. Races 0 and 1B/C causes a yellowing syndrome and races 1A, 2, 3, 4, 5 and 6 induce a wilting syndrome (Jimenez-Gasco and Jimenez-Diaz, 2003). Highly susceptible plants wilt 25 days after sowing in the field and drooping of the leaves and paler colour are the symptoms of Foc infection. Adult plants show gradual fading of leaf colour and drooping of rachis and petioles. Roots show no external rotting, drying or discolouration but vertical section of root shows internal discolouration of pith and xylem (Ali *et al.*, 2012). *Fusarium* wilts in general are warm soil diseases and very severe at 28°C or near the optimum temperature for growth of the pathogen in culture. Eventhough temperature appears to be the most important factor, light and nutrients also effect *Fusarium* wilt development either solely or by interacting with temperature. Increased moisture was associated with increased wilt severity.

Marker assisted selection (MAS) based on the use of DNA markers linked closely to wilt resistance genes can be used to screen large number of chickpea breeding lines for presence of these genes without actually subjecting them to the pathogen. Thus the process hastens identification of resistant genotypes. DNA-based diagnostic assays which are fast and being developed for the pathogen and its races do not need screening of differential lines and are not influenced by environment (Jimenez-Gasco and Jimenez-Diaz, 2003). The major breakthrough, in the development of polymorphic markers was the identification of microsatellite based markers. Consequently a number of STMS, RAPD, SCAR and ISSR markers linked to various wilt resistance genes were identified. The markers in the present study were selected for molecular screening on the basis of their linkage with resistance genes for four different races *viz*. 1, 2, 4 and 6 of the pathogen. TA110 has been found to be linked to *foc-1* race of wilt by a distance of 2.1 cM, TA59 to *foc-1, foc-3* and *foc-4* races of wilt at the distances of 4.4 cM, 0.5 cM and 3.8 cM, respectively (Benko-Iseppon *et al.,* 2003). TA96 has been reported to be linked with four races of wilt *viz*. *foc-1, foc-2, foc-3* and *foc-4* by the genetic distance of 4.9 cM, 1.5 cM, 0.5 cM and 3.3 cM, respectively (Rubio *et al*., 2003)]. The marker TA27 is reported to be linked to *foc-1, foc-2* and *foc-3* races of *Fusarium* at the distances of 4.9 cM, 1.5 cM and 0.5 cM, respectively (Sharma *et al*., 2004). TA59 linked to *foc-1*, *foc-3, foc-4* and *foc-5* at the distances of 4.4, 0.5, 3.8 and 2.4 cM respectively (Gowda *et al*., 2009). The first wilt resistance gene to be tagged in chickpea was H1 (Mayer *et al.,* 1997). The gene was 7.0 cM from UBC-170_{550}. Marker $A_{07}C_{417}$ is reported to be linked with allele for susceptibility to *foc-1* at a genetic distance of 0.4 cM (Sharma and Muehlbauer, 2005). Marker CS27A was linked with susceptibility allele at H1 locus for race 1A (Soregaon and Ravikumar, 2010). Marker UBC-825_{1200} was linked to the gene for resistance to *Fusarium* wilt race 4 (Ratnaparkhe *et al*., 1998). In the present study phenotypic screening of six genotypes for wilt reaction under sick-pot conditions in green house and in unprotected field conditions were undertaken. Also, markers formerly reported to be linked with genes conferring resistance to different races were used for molecular screening of six chickpea genotypes. Finally identification and evaluation of chickpea genotypes aiming at to combine phenotypic screening linked with gene using PCR based markers was made (Ahmad *et al.,* 2014).

MATERIALS AND METHODS

Plant Material

Six chickpea genotypes namely, PG043, HC1, DCP92-3, GNG1581, PKG-024 and PG071 were taken for artificial screening against *Fusarium* wilt and markers validation. All genotypes were sown in sick pots for artificial screening. Salient features of different chickpea genotypes given in table1.

Screening against Wilt under Artificial Conditions in Sick-Pots

Six genotypes were screened for their reaction against four different races of wilt *viz*., 1, 2, 4 and 6 under greenhouse conditions at 25^{o}C temperature and 40% relative humidity. Cultures of *F. oxysporum* f. sp. *ciceris* isolates for each of four races were obtained by placing small aliquots (approximately 100 mg) of a soil culture of each isolate onto a plate of potato dextrose agar (PDA) (250g of unpeeled potatoes, 20g of agar, and 20g of glucose per liter of distilled water) and incubating the plate for 10 days at 25^{o}C with a 12-h photoperiod of fluorescent and near-UV light at 36 µE $m^{-2}s^{-1}$. Inocula for experiments were produced in an autoclaved cornmeal media (20g of corn meal, 20g of peptone, 20g dextrose per liter of distilled water) in flasks incubated under the same conditions for 2 wks. The infested corn meal media was mixed thoroughly with soil mixture pre-exposed to intense sunlight for a long period, at a rate of 1:12 (wt/wt) and incubating the mixture in polyethylene bags for 7 days under the same conditions. The ratio of infested corn meal in the

mixture ensured an inoculum density of approximately 10^5 CFU/g soil. Infested soil mixtures for each four races were then mixed thoroughly and uniformly to the soil to be filled in the pots. Each of six genotypes in combination with each of four races (1, 2, 4 and 6) was planted with three replications, during *rabi* season 2013-14. The experimental pots for each genotype consisted of 9 seeds/pot. All recommended package of practices were followed for raising a good chickpea crop.

OBSERVATIONS RECORDED

At seedling and reproductive/physiological maturity stage disease were identified and data was recorded for per cent wilting in different genotypes for different races. The percentage of wilt incidence of each entry was calculated by following formula:

Wilt incidence = Number of wilted plants/Total number of plants x100

The level of resistance and susceptibility of each test entry was determined by using 1-9 disease rating scale given by Iqbal (2005), where 1=highly resistant (0-10% plants wilted), 3=resistant (11-20% plants mortality), 5=moderately resistance (21-30% mortality), 7=susceptible (31-50% mortality) and 9= highly susceptible (more than 50% mortality).

DNA Isolation

The genomic DNA from each genotype was isolated from young leaves of 20 days old seedlings grown in the sick pots. DNA was extracted from genotypes using CTAB (cetyl trimethyl ammonium bromide) method as described by Saghai-Maroof *et al.*, (1984). The quality and quantity were estimated by measuring O.D. at 260/280 and 260 nm, respectively in a spectrophotometer. Intactness of genomic DNA was checked on 0.8% agarose gel.

PCR Amplification and Electrophoresis

The sequence information and numbers of bases (mers) of the primers taken for present study are given in Table 6. Amplifications were performed in a 12.34 µl reaction mixture containing 1.5 µl Taq buffer 1X containing [10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$], 0.32 mM of dNTPs, 0.75 µl of each forward and reverse markers of 0.05 µg/µl, 50 ng genomic DNA and 1 U/µl Taq DNA polymerase. The temperature profile for STMS markers consisted of an initial denaturation step of DNA at 94°C for 4 min, followed by 35 cycles: 94°C for 2 min, 55 to 58°C for 50s and 72°C for 50s. Annealing temperatures were optimized individually for each marker. After the final cycle, samples were incubated at 72°C for 7 min to ensure complete extension followed by hold at 4°C. For RAPD markers reactions were performed in the same thermal cycler with same reaction volume but thermal cycler was programmed for a first denaturation step for 5 min at 95°C; followed by 40 cycles of 1 min at 94°C, annealing for 50s at 30-36°C and extension for 2 min at 72°C. Annealing temperatures were optimized individually for each marker. For SCAR marker thermal cycler was programmed as initial denaturation step for 4 min at 94°C; followed by 40 cycles of 20s at 94°C, annealing for 1 min at 64°C and extension for 1 min at 72°C. The temperature profile for ISSR marker consisted of an initial denaturation step at 95°C for 5 min, followed by 40 cycles: 94°C for 1 min, 45°C for 1 min and 72°C for 1 min. PCR amplified products were mixed with 4 µl of 6X loading dye (0.25% bromo-phenol blue, 0.25% xylene cynol and 40% sucrose, w/v), electrophoresed on 1.5 or 2.5 % agarose gel, stained with ethidium bromide and visualized under UV light.

Scoring of Amplified band

The amplified products were visualized under UV light and photo-graphed by Bio-Rad gel documentation system for each marker. The PCR products for marker analysis were scored for fragment length polymorphism.

RESULTS AND DISCUSSIONS

Artificial Screening

Table 2 showed numbers of wilted plants of six different genotypes of chickpea against four different races (1, 2, 4 and 6) whereas Table 3 showed above all percent wilted plants of six genotypes at two stages *i.e*., at seedling stage and reproductive stage. Variation was observed between genotypes for their disease reaction at both the stages (Table 4). Out of six genotypes, one genotype PKG024 showed highest 23% and 59% of wilted plants at both seedling and reproductive stages, respectively. This was followed by genotype DCP92-3 with 20% and 25% of wilted plants at seedling and physiological maturity stages, respectively. No wilting was observed in genotype GNG1581 at seedling stage and only 3% wilting at maturity stage. Overall disease reaction of six genotypes at two stages was given in Table 4. At seedling stage, genotypes PG043, HC1, GNG1581 and PG071 were found to be highly resistant where DCP92-3 scored as resistant and PKG024 showed moderately resistant response. At reproductive/physiological maturity stage one genotype GNG1581 was highly resistant, two genotypes PG043 and PG071 were resistant, two genotypes HC1 and DCP92-3 were moderately resistant and one genotype PKGO24 was scored as highly susceptible. The disease incidence at physiological maturity stage increased invariably in some genotypes as compared to that at seedling stage. On an average basis 90% disease resistance was recorded at early stage and 77% at reproductive stage or in other words, 10% disease incidence was observed at seedling stage and 23% at reproductive stage. Almost, all the genotypes showed an increase in disease incidence at physiological maturity stage as compared to seedling stage. This means that such genotypes required long wilting time. Since resistant lines at seedling stage also became susceptible at reproductive stage, so screening at reproductive stage seems to be more reliable. Ahmad *et al.* (2007) evaluated 139 genotypes obtained from various sources to identify disease resistance against *Fusarium* wilt under artificial control conditions and considerable variation among genotypes was observed at seedling and reproductive stages. Sarwar *et al.* (2012) tested chickpea lines for resistance potential against *Fusarium* wilt in wilt sick field and observed significant difference for both early and late season wilt incidence.

Natural Screening

Six different genotypes were evaluated in unprotected field conditions against the prevalent races of *Fusarium* at reproductive stage due to the prevalence of disease for a short period at seedling stage and for a long period at the reproductive stage. Since high temperature plays an important role for the development of disease and the high temperature prevailed for a short period at seedling stage due to onset of the winter in December and it prevailed for a long time at reproductive stage due to the onset of summer at the time of flower initiation. Therefore, disease prevailed for a longer time at reproductive stage of observation (Ahmad *et al.,* 2010). The results revealed different response of various genotypes for wilt disease. During screening, out of six genotypes, PKG024 showed highest wilting of 42 % followed by DCP92-3 with 11 % wilting. Remaining four genotypes namely, PG043, HC1, GNG1581 and PG071 showed 8, 9, 2 and 7 % wilting respectively. Therefore three genotypes namely, PG043, GNG1581 and PG071 were categorized as highly resistant, DCP92-3 as resistant and PKG024 fell under the category of susceptible genotype. Sidramappa *et al.* (2010) also evaluated 126 recombinant inbred lines (RILs) derived from a cross ICCV-2 X JG-62 along with six checks for wilt resistance under unprotected natural infestation and classified genotypes as highly resistant, moderately resistant,

intermediate, susceptible and highly susceptible based on percent plants wilted. Variations between field and sick pots results were observed for some genotypes for their wilt reaction, this may be due to the fact that race 1 is not found in India and race 6 is not prevalent race in Pantnagar, and less level of disease incidence in field conditions may be due to the fact that crop often has the chances of disease escape as the wilt disease is temperature dependent and the level of inoculum may vary at different places and insufficient inoculums density in the field for wilt to occur. The resistance source of *Fusarium* wilt in chickpea germplasm is not uncommon and a number of other workers have also reported the occurrence against high level of resistance to *Fusarium* wilt (Iftikhar *et al*., 1997).

Molecular Screening and its Correlation with Artificial Screening

To further evaluate and identify wilt resistant chickpea genotypes different markers were investigated to assess linkage with *Fusarium* wilt resistance genes (Table 5). A high level of resistance in chickpea genotypes against *Fusarium* wilt disease has been studied. But identification and evaluation of chickpea wilt resistant lines aiming at to combine field screening linked with gene using PCR based markers is a new avenue in chickpea breeding in Pakistan (Ahmad *et al.*, 2014). The marker TA110 has been reported to be linked with race 1 by a genetic map distance of 2.1 cM. It amplified two bands in all the genotypes, lower band in the range of 221-233 bp and upper band ranging between 481-510 bp in size (Fig.1). Four genotypes namely, PG043, DCP92-3, GNG1581 and PG071 that showed highly resistant and HC-1 resistant reaction to the race 1 in artificial screening, were found to have TA110 marker associated with an allele for resistance. PKG024 which was reported susceptible in artificial screening had marker allele size of 231 and 481 bp for susceptibility. Ali *et al*. (2012) evaluated eleven lines of chickpea against different races of *Fusarium*, where TA110 amplified a marker size of 320 bp in resistant genotypes and 210 bp in susceptible genotypes. With markers UBC-170, $A_{O7}C_{417}$, $CS27A_{700}$ and UBC-825, genotype PKG024 amplified band of size 550, 710, 700 and 1193 bp respectively. The alleles of these sizes were amplified in genotype which was susceptible to all races *i.e.* PKG024 only, while absent in other genotypes. However, variation was observed with genotype DCP92-3 which showed susceptibility to race 4, but it does not amplified susceptible allele with markers UBC-170 and CS27A. However, the UBC-825 was able to give amplification in this genotype with band size of 1190 bp. This variation in disease reaction may be due to large distances of markers with resistant gene for race 4. Padaliya *et al.* (2013) used marker UBC-170 linked to susceptibility for characterization of six chickpea genotypes and also observed that RAPD marker UBC-170 gave product of 550 bp in susceptible genotypes only. Soregaon and Ravikumar (2010) used primer $A_{07}C_{417}$ linked to the allele for susceptibility at H_2 locus of *Fusarium* wilt and characterized wilt resistance in different chickpea genotypes, where presence of $A_{07}C_{417}$ indicated susceptible early wilting. Padaliya *et al.* (2013), evaluated six chickpea genotypes for *Fusarium* wilt reaction using marker CS-27A and UBC-825 that amplified product of 700 bp and 1200 bp in susceptible genotypes respectively, whereas amplification was absent in other genotypes. TA59 has been reported to be linked to four races of wilt *foc-1, foc-3, foc-4* and *foc-5* by the genetic distance of 4.4, 0.5, 3.8 and 2.4 cM, respectively. The primer TA59 showed polymorphic bands among the genotypes. Single band in the range of 210 to 309 bp was obtained in six genotypes. The largest amplicon size of 309 bp was observed in GNG1581 while smallest amplicon size of 209 bp was obtained in the genotype HC1. We obtained incongruent result on genotype HC1 with this marker as this line showed resistance phenotype for race 1 and moderately resistant to race 4 but had a TA59 marker associated with an allele for susceptibility. Such a contradictory result in association of the marker with the *foc1* and *4* phenotypes might be due to recombination like event (Ali *et al.*, 2012) or insufficient inoculum density in sick pot for disease to occur. The PCR amplification of TA59 primer by Padaliya *et al.* (2013) generated two alleles, out of which the allele of 258 bp was present only in resistant genotypes. Markers TA96 and TA27 have been reported to be

linked with five races of wilt *foc-1, foc-2, foc-3, foc-4* and *foc-5* by the genetic distance of 4.9, 1.5, 0.5, 3.3 and 2.9 cM, respectively. So, artificial screening for three races *viz.* 1, 2 and 4 were correlated with molecular results. The primer TA96 amplified polymorphic bands in the six genotypes. The genotypes PG043 and PGO71 amplified two bands, genotype PG043 amplified bands of size 200 and 270 bp and PG071 gave amplicons of size 230 and 281 bp. Remaining genotypes *viz*. HC1, DCP92-3, GNG1581 and PKG024 amplified only one band of sizes 273, 275, 278 and 260 bp, respectively. The primer TA27 showed two bands in all the genotypes. Lower band varied between 103-109 bp and upper band varied between 250-260 bp in size. The large upper band size of 255 bp and 260 bp was obtained in two genotypes PG043 and PG071, respectively. One genotype PKG024 showed minimum lower band of size 103 bp and minimum upper amplicons of size of 180 bp. Genotypes GNG1581, DCP92-3 and HC1 showed upper band of sizes 250, 253 and 257 bp, respectively. Sharma *et al*. (2004) reported similar results in evaluation of 22 lines of chickpea against wilt and size of the bands amplified by TA96 in different genetic backgrounds varied from 250 bp to 306 bp. They also found the genotypes with largest band size of 306 bp as resistant and with smallest band size of 250 bp as susceptible ones. Similarly, Gowda *et al.* (2009) validated TA96 primer using 16 diversified genotypes and they observed ten of the thirteen genotypes carrying resistance associated allele to *foc-2.* STMS primer TA96 was utilized by Padaliya *et al*. (2013) to characterize chickpea genotypes for *foc-4* resistance, the alleles of 265 bp amplified by primer TA-96 was present only in resistant genotypes; whereas the same was absent in other genotypes. Sharma *et al.* (2004) revealed that primer TA27 amplified a band of 241 bp in resistant RILs and 235 bp in susceptible RILs. Again it varied from 201 bp (*C. reticulatum*, susceptible) to 273 bp (DZ10-4, resistant). Ali *et al.* (2012) evaluated eleven lines against different races of *Fusarium* using different markers, where TA27 amplified a marker size of 249 bp in susceptible genotypes and gave a product of 255 bp in resistant genotypes. This little variation in band size may be due to different genetic background. The pathogenicity test for five genotypes against races 1 and 2 were in accordance with marker data for TA96 and TA27 but one genotype DCP92-3 showed susceptible phenotype for race 4 but had a marker associated with an allele for resistance. Genotypes evaluated against race 6 of *Fusarium oxysporum* f. sp. *ciceris* were not correlated with molecular data as genes conferring resistance to race 6 has not been mapped (Sharma and Muehlbauer, 2007). So, in the present study we obtained incongruent result on chickpea genotype DCP92-3 that showed susceptible reaction from artificial screening against race 4, but was determined to carry the allele associated with resistance for markers TA59, TA96 and TA27, on the other hand it did not amplified susceptibility associated with markers UBC-170, CS27A and UBC-825. It means incongruent result is due to ineffective screening in greenhouse for the reaction of DCP92-3 to race 4. Another variation was observed with HC1 which showed highly resistant reaction to race 1, but carried the susceptibility associated with TA59. It can be concluded that genotypes PG043, GNG1581 and PG071 were found to be resistant for three races 1, 2 and 4, while PKG024 was found to be susceptible after phenotypic screening and molecular characterization with all the linked markers. The MAS enhances sources of distinction and make easy the complex trait selection that is otherwise time consuming process when evaluated phenotypically. The present study selected wilt resistant genotypes using various markers can provide an opportunity in marker assisted breeding.

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APPENDICES

Table 1: List of Chickpea Genotypes and their Sources/Parentage

Sr. No.	Genotype	Sources/Parentage
1.	PG043	K-850 (LM) X Avrodhi
2.	HC1	-
3.	DCP92-3	Selection from local germplasm
4.	GNG1581	GPF2 X H82-2
5.	PKG024	PG92-105 X Pusa362
6.	PG071	BG1053 X PKC-1

Table 2: Wilt Incidence of Six Chickpea Genotypes at two Stages against Four Races of *Fusarium*

Genotype	Race 1		Race 2		Race 4		Race 6	
	S	PM	S	PM	S	PM	S	PM
PG043	0/20	2/20	1/21	2/21	2/21	6/21	0/21	1/21
HC1	1/21	4/21	3/22	4/22	4/23	7/23	3/23	6/23
DCP92-3	2/21	2/21	3/23	5/23	8/24	9/24	5/23	7/23
GNG1581	0/21	0/21	0/23	2/23	0/21	1/21	0/25	0/25
PKG024	5/23	11/23	3/21	9/21	9/21	19/21	3/21	12/21
PG071	0/25	0/25	0/21	3/21	1/24	3/24	0/21	1/21

S-Seedling stage, PM-Physiological maturity stage

Wilt Incidence: number of plants wilted/ total number of plants screened

Table 3: Percent Wilt Incidence of Six Chickpea Genotypes at two Stages in Artificial Screening

Genotype	Total No. of Plants	No. of Plants Wilted		% of Plants Wilted	
		Seedling Stage	Physiological Maturity Stage	Seedling Stage	Physiological Maturity Stage
PG043	83	3	11	4	13
HC1	89	11	21	12	23
DCP92-3	91	18	23	20	25
GNG1581	90	0	3	nil	3
PKG024	86	20	51	23	59
PG071	91	2	11	2	12
	530	**54**	**120**	**10**	**23**

Table 4: Disease Rating of Six Genotypes at two Stages for Wilt Reaction in Artificial Screening

Disease Scores	Seedling Stage	Reproductive Stage
1- Highly Resistant	PG043, HC1, GNG1581 and PG071	GNG1581
3- Resistant	DCP92-3	PG043 and PG071
5- Moderately Resistant	PKG024	HC1 and DCP92-3
7- Susceptible	-	-
9- Highly Susceptible	-	PKG024

Table 5: Disease reaction of Different Chickpea Genotypes to *Foc* Races 1, 2, 4 and 6 and Marker Characterization

Genotype	Race1	Race 2	Race 4	Race 6	TA110	TA59	TA96	TA27	UBC-170	$A_{07}C_{417}$	CS27A	UBC-825
PG043	HR	HR	MR	HR	R	R	R	R	R	R	R	R
HC1	R	R	MR	MR	R	S	R	R	R	R	R	R
DCP92-3	HR	MR	S	S	R	R	R	R	R	R	R	R
GNG1581	HR	HR	HR	HR	R	R	R	R	R	R	R	R
PKG024	S	S	HS	HS	S	S	S	S	S	S	S	S
PG071	HR	R	R	MR	R	R	R	R	R	R	R	R

HR=Highly Resistant, R=Resistant, MR=Moderately Resistant, S=Susceptible, HS=Highly Susceptible

Table 6: **Characteristics of Four STMS, Two RAPD, one SCAR and One ISSR Markers**

Sr. No.	Primer Name	Primer Sequence (5'- 3')	MERS
	STMS		
1.	TA110	F-ACACTATAGGTATAGGCATTTAGGCAA	27
		R-ACACTATAGGTATAGGCATTTAGGCAA	27
2.	TA59	F- ATCTAAAGAGAAATCAAAATTGTCGAA	27
		R- GCAAATGTGAAGCATGTATAGATAAAG	27
3.	TA96	F- TGTTTTGGAGAAGAGTGATTC	21
		R- TGTGCATGCAAATTCTTACT	20
4.	TA27	F-GATAAAATCATTATTGGGTGTCCTTT	26
		R- TTCAAATAATCTTTCATCAGTCAAATG	27
	RAPD		
5.	UBC-170	ATCTCTCCTG	10
6.	$A_{07}C_{417}$	GAAACGGGTGC	11
	SCAR		
7.	CS27A	F-AGCTGGTCGCGGGTCAGAGGAAGA	24
		R-AGTGGTCGCGATGGGGCCATGGTG	24
	ISSR		
8.	UBC-825	ACACACACACACAC	15

F=Forward Primer,R=Reverse Primer, Mers= Number of bases

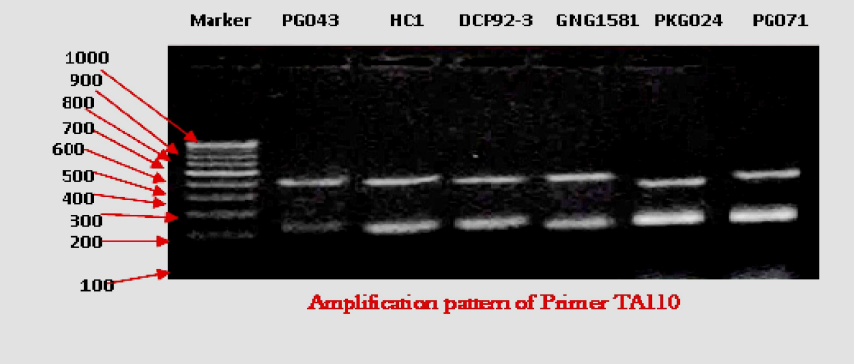

Figure 1: Amplification Pattern of Marker TA110